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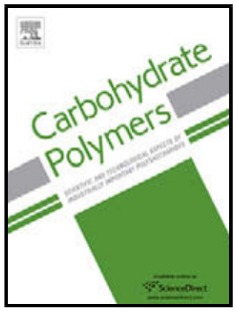

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Microwave radiation and protein addition modulate hydration, pasting and gel rheological characteristics of rice and potato starches

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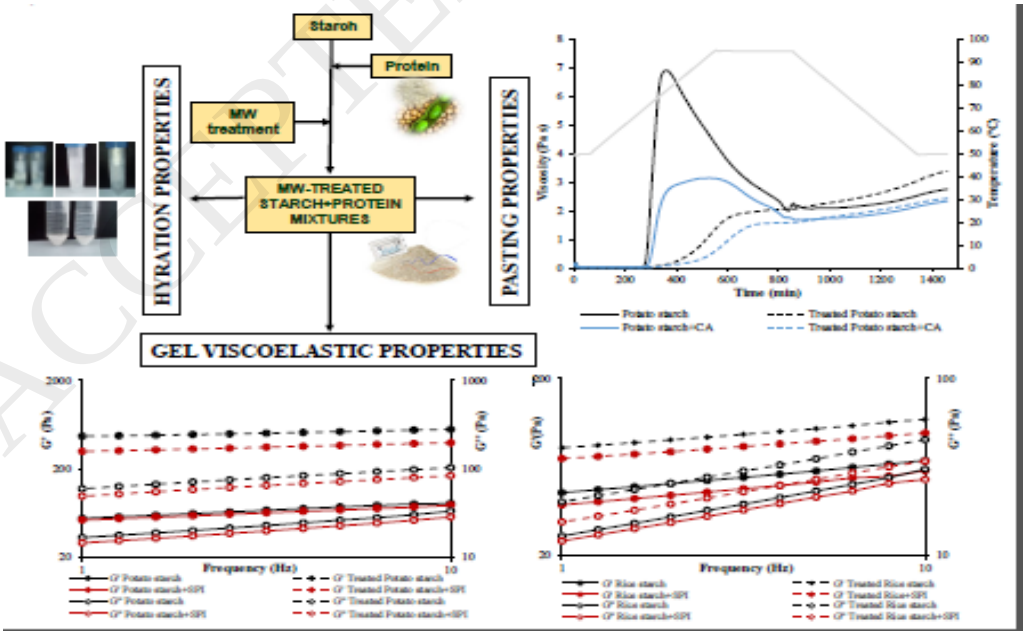
Graphical abstract



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Highlights:

- The swelling power of potato starch increased after the microwave treatment
- Microwave treatment decreased the swelling power of rice starch
- The protein presence changed the impact of microwaves on starch properties
- Microwave radiation altered the pasting properties of potato starch
- Microwave treatment increased viscoelastic moduli of potato starch gels

Abstract

This study evaluated for first time the effect of Microwave (MW) radiation on systems based on potato and rice starches supplemented with 5% of calcium caseinate (CA) or soy protein isolate (SPI). The goal of this treatment was the physical modification of these starch-based systems to provide ingredients of new functionalities. The hydration and pasting properties as well as gel viscoelastic features were evaluated. Dynamic oscillatory rheological tests were used. The effect of MW treatment (MWT) depended on the starch botanical origin and was significantly affected by protein presence and type. MWT of starch+protein blends revealed the most notable changes when SPI was added. Adding it to rice starch decreased swelling power (-45%), altered viscometric profiles and reinforced gel structure with important increases in both viscoelastic moduli (+160%-G' and +58%-G''). In blends with potato starch, MWT increased water absorption capacity (+115%), and decreased water solubility index (-82%). MWT of protein-potato blends promoted gel stability, decreased their pasting profiles and resulted in enhanced viscoelastic moduli (+483-G' and 243%-G''). MWT combined with protein addition allows designing starch-based foods with tailored properties.

Keywords: Microwave; functional properties; gel rheology; potato starch; rice starch

1. Introduction

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The native form of starch has limited applications in the food industry and is often modified physically, chemically or enzymatically to improve functional properties. Physically modified starch is considered a natural material and a highly safe ingredient, much safer and more predictable than chemically modified starch. So far, the usage of physically modified starch in food is not restricted by legislation, which is considered an outstanding advantage compared to chemically- or enzymatically-modified starch applications (Klein et al., 2013).

Starch, as a macro-constituent of many raw materials and processed foods, contributes to their nutritional and functional characteristics, as well as plays the pivotal role as a texture modifier (Walkenström and Hermansson, 1998) and influences many quality attributes of processed foods (Chen et al., 2015). Rice and potato starches are among the most commonly used for texture development, particularly in gluten-free (GF) products. During microwave (MW) radiation exposure, energy is delivered directly to the material, heating the entire sample in bulk at a faster heating rate than conventional heating (Bilbao-Sáinz et al., 2007). Therefore, MW radiation-induced heating is suitable for modifying starches physically and improving their functional properties. In moistened starches, the intermolecular structure changes from MW-delivered energy lead to changes in water absorption ability, solubility and swelling power, as well as in starch gelatinization, syneresis and paste viscosity (Brasoveanu and Nemtanu, 2014). There are many factors related to both starch characteristics (botanical origin, water content, density, dielectric properties, etc.) and microwave irradiation conditions (frequency, power and exposure time) that significantly affect how granular starch responds to MW treatment (MWT) (Brasoveanu and Nemtanu, 2014). Zavareze et al. (2010) showed that microwave irradiation affected the pasting properties of high-amylose rice starches more intensely than in those with lower amylose content.

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Vermeylen et al. (2006) concluded that higher treatment temperatures and moisture contents generally caused the largest changes in starches. As applied temperature and microwave power level increased, the peak viscosity of potato starch decreased (Nadiah et al., 2015), while the gelatinization temperature increased (Nadiah et al., 2015; Vermeylen et al., 2006). Villanueva et al. (2018a) concluded that the MW radiation absorption capacity of rice flour exhibited a positive quadratic relationship with water content, while the flour dry matter did not show any radiation absorption capacity. They also observed changes in physical properties of treated rice flour, such as rice flour particle morphology or the crystallinity/amorphous region ratio.

Beyond physico-chemical and functional considerations, starchy gluten-free foods are often considered nutritionally poor compared to wheat-based counterparts due to their deficient protein/starch ratio (Villanueva et al., 2015). The addition of protein is a viable alternative to improve the nutritional properties of these products. Proteins are also known to modify structure and texture in foods (Villanueva et al., 2018b). Soy proteins isolate (SPI) and calcium caseinate (CA) have been investigated in GF applications because of their effect on nutrition and structure of products (Krupa-Kozak et al., 2011; Ronda et al., 2014). Starch-protein interactions affect the rheological, pasting, gelatinization, textural and physicochemical properties of food systems (Gallagher et al., 2003; Ronda et al., 2011; Ronda et al., 2014; Villanueva et al., 2015). As far as we know, how MWT affects protein-starch mixtures has not been studied so far, in spite of how important endogenous proteins seem to be in the effect of heat-moisture treatment (HMT) on treated-flour properties (Puncha-arnon and Uttapap, 2013). As protein and starch blends can serve as model systems for the study of more complex matrices such as flour, the impact of their interactions on changes appearing in MW treated samples needs to be better understood. The main objective of this study was to investigate the

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combined effect of microwave radiation and protein addition (5% CA or SPI) on the hydration, pasting properties and gel viscoelastic properties of potato and rice starches.. The ultimate goal of this study is to provide starch-based ingredients with tailored functional properties.

2. Material and methods

2.1. Materials

Rice starch and potato starch were supplied by Ferrer Alimentación S.A. (Barcelona, Spain). Soybean protein isolate (SPI) Supro 500-E IP (purity ~ 90%) was obtained from Proveedora hispano-holandesa S.A. (Barcelona, Spain), and calcium caseinate (CA) (purity ~ 96%) from Armor Protéines (Saint-Brice-en-Coglès, France). Protein added in all the starch+protein blends was 5 g/100 g blend. Twelve different samples were investigated: native and MW-treated rice and potato starches alone and blended with 5 g/100 g of CA or SPI proteins.

2.2. Microwave treatment of starch samples

Samples were treated by MW radiation at initial moisture content of 30%. The l moisture content of the samples was measured following the American Association of Cereal Chemists (AACC) 44–19 method and the water necessary to adjust to 30% moisture content was sprayed onto the samples while they were mixed in a Bear Teddy Mixer (Bear 5L Teddy, Swansea, UK) for 15 min. Samples were then hermetically sealed in bags and held 24 h at 4 ± 2°C for the moisture equilibration.

Hydrated samples (100.0 g each) were heated in a SHARP R-342 (Osaka, Japan) microwave oven in a cylindrical polyethylene container closed with needle-punched, microwave-safe food grade film. The homogenous distribution of radiation on the sample is of crucial importance. So, the container was stirred constantly to ensure a uniform energy and temperature distribution during treatment as described in

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Villanueva et al. (2018b). MW radiation frequency was 2450 MHz and the power, 900W. MW was applied in cycles of 20 s radiation with 40 s downtime, for a total of 32 min and 640 s of MWT; afterwards, samples were left to cool for 5 minutes. Agglomerates formed during treatment were manually disintegrated in a laboratory mortar to < 0.5 mm. Sample temperature during MWT was measured with Testoterm thermometer strips of different scales, and 0.5ºC accuracy (Instrumentos Testo S.A., Barcelona, Spain), as described in Villanueva et al. (2018a). Each measurement was made in duplicate. The temperature evolution curves obtained for the potato and rice starch systems studied were equivalent to those reported for 30% moistened-rice flour in our previous work (Villanueva et al., 2018a). The temperature reached and maintained after 4 min of treatment was 157±5ºC for all samples.

2.3. Amylose content

The amylose content of the starch samples was determined using a colorimetric assay kit for amylose/amylopectin ratio determination in starch (Megazyme International Ireland Ltd., Ireland), according to the manufacturer's procedure based on Gibson et al., (1997) method. The method determines the soluble amylose in acetate/salt solution susceptible to α-amylase/ amyloglucosidase digestion. Each sample was analysed at least in duplicate.

2.4. Hydration properties

Water absorption capacity (WAC) of the samples was determined by the centrifugation method described by Abebe et al., (2015). Two grams of sample (w_s) were mixed with 20 mL of distilled water in 50 mL centrifuge tubes. The dispersions were hold at room temperature for 30 min with occasionally vortexed (Heidolph Reax, Schwabach, Germany) and followed by centrifugation for 30 min at 3000×g (Thermo Fisher Scientific, Waltham, USA). The supernatant was removed and weighed (w_{s+w}) and

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results were expressed as grams of water retained per gram of sample. Water absorption index (WAI) and water solubility index (WSI) were measured with slight modification of the method used by Abebe et al. (2015). Each sample of 2.5 g (w_0) was dispersed in 30 ml of distilled water in tared centrifuge tubes. After cooking for 10 min in a 90ºC water bath, samples were cooled to room temperature and centrifuged at 4000xg for 10 min. The supernatant was poured into a pre-weighed evaporating capsule to determine the solid content and the sediment was weighed (w_{ss}). The weight of soluble solids was recovered by evaporating the supernatant overnight at 110ºC (w_{ds}). WAC, WAI, WSI and swelling power (SP) were calculated using the following equations:

$$WAC\left(\frac{g}{g}\right) = \frac{w_{s+w} - w_s}{w_s} \qquad (1)$$

$$WAI\left(\frac{g}{g}\right) = \frac{w_{ss}}{w_0} \qquad (2)$$

$$WSI\left(\frac{g}{100g}\right) = \frac{w_{ds}}{w_0} \times 100 \qquad (3)$$

$$SP\left(\frac{g}{g}\right) = \frac{w_{ss}}{(w_0 - w_{ds})} \qquad (4)$$

All results were referred to dry matter to avoid the effect of different water content in the samples.

2.5. Thermoviscous test: Viscometric profile

Following AACC International Method 76-21.01 Standard 2, viscometric profiles of MW-treated and untreated samples were obtained using a Kinexus Pro+ rheometer (Malvern Instruments Ltd., Malvern, UK) supplied with starch pasting cell and controlled by rSpace software. Samples of rice starch (3 g, 14% moisture-based) or potato starch (2 g, 14% moisture-based) with or without added protein were transferred to a canister where 25 mL±0.1 mL of distilled water was added. Each starch suspension was equilibrated at 50 ºC for 1 min, heated to 95 ºC at a rate of 6 ºC/min, maintained at 95 ºC for 5 min, then cooled to 50 ºC at a rate of 6 ºC/min, and maintained at 50 ºC for 2

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min. Paddle speed was set at 960 rpm for the first 10 s and then 160 rpm for the rest of the analysis. Each sample was analyzed at least in duplicate. Parameters calculated from the pasting profiles were pasting temperature (PT), peak viscosity (PV), trough viscosity (TV), breakdown (BD), final viscosity (FV), setback (ST) and peak time.

2.6. Rheological measurements

Dynamic oscillatory tests of the twelve potato and rice starch gels were performed with Kinexus Pro+ rheometer (Malvern Instruments Ltd., Malvern, UK) with parallel plate geometry (40 mm diameter) of serrated surface and with 1 mm of working gap. The gel samples were made following the same procedure described for thermoviscous tests. Different concentrations of rice and potato starches were used to prepare their gels, so that the gels produced from native starches were of similar consistencies. Just at the end of the pasting test the gel was removed from the canister and placed between the plates, the sample excess was removed and the sample was left to rest for 5 min to allow relaxation. Temperature was stabilized at 25ºC with a Peltier plate controller. Stress sweeps were performed from 0.1 to 500 Pa at a constant 1 Hz frequency. Frequency sweeps were carried out from 10 to 1 Hz in the linear viscoelastic region (at a constant value of 1 Pa). Frequency sweep data were fitted to potential equations as described by Ronda et al. (2014). All gels were prepared at least in duplicate and rheological tests were also performed in duplicate.

2.7. Statistical analysis

Statgraphics Centurion v.6 (Bitstream, Cambridge, MN, USA) was used for multifactor analysis of variance (ANOVA) of the data. The Least Significant Difference test was used to evaluate significant differences ($p < 0.05$) between samples.

3. Results and discussion

3.1. Amylose content

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The amylose/amylopectin ratio of starch, which greatly affects starch functional properties, is attributed to different factors such as botanical source, soil type and climatic conditions during plant growth (Jane et al., 1999; Singh et al., 2006). The amylose contents in our native samples were 16±1% and 20±1% for rice and potato starch, respectively. MWT reduced the amylose content in the potato starch to 15±1% (significantly, $p<0.05$), while no change was observed for rice starch. This result is coherent and confirms that amylose-amylopectin (AM-AMP) interactions promoted by MW-assisted HMT reduced amylose solubility of treated potato starch, as previously reported by Varatharajan et al. (2010). The method used for amylose quantification includes a previous separation step with Concanavalin-A (Con-A) that specifically complexes branched polysaccharides of amylopectin starch components (Gibson et al., 1997). The AM-AMP interaction could lead to a partial co-precipitation of amylose, leading to a reduction in the final amylose content. These interactions must have been weaker or less efficient in rice starch, given that amylose content results were hardly affected by MWT compared to potato starch. The same happened with the other properties measured in the treated mixtures, as shown below.

3.2. Hydration properties

Hydration properties were affected by all the factors studied: starch and protein type as well as MW treatment (see Table 1). WAC depends on starch structure, the degree of association to form hydrogen bonds between starch chains, internal forces controlling granule structure and availability of water binding sites (Gani et al., 2017). This would justify the differences found between the different native starches tested. Rice starch WAC was 26% higher than that of potato starch. The addition of 5% SPI to native starches improved their water retention properties and increased potato starch WAC by up to 42%. Similar results were reported by Chinma et al. (2013) when SPI was added

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to cassava starch. However, adding CA had no significant effect ($p>0.05$) on the WAC of the blends regardless of the starch to which it was added. Protein characteristics have been found to influence functional behaviour (Cornejo & Rosell, 2015). Polar amino acids have been shown to be primary sites for protein interaction in water, so the increased availability of these amino acids in SPI may explain the WAC increases observed when this protein was added (Li et al., 2010). WAC increased 37% in MW-treated rice starch and 117% in MW-treated potato starch (Table 1). High temperature applied to the moistened sample during MWT yields a high level of damaged starch (Pinkrova et al., 2003). This would explain the water absorption capacity increase. In addition, the internal granule structure collapses and the crystallites are disrupted during HMT (Hoover, 2010); this could justify the fact that the starches uptake more water. MWT had no effect on rice starch WAC in presence of proteins, while a significant effect on potato starch was observed. Especially for SPI, WAC increased ~200% with respect to native protein-free potato starch.

Water absorption index (WAI) and swelling power (SP) values are shown in Table 1. These parameters depend on the interaction between starch chains within the amorphous and crystalline domains; amylose and amylopectin content, molecular weight distribution and branching length and degree, phosphate groups and starch molecule conformation all influence WAI and SP (Ratnayakea et al., 2002). The difference in tuber and cereal starch structures, especially the higher amylose and monoester phosphate content in tubers, explains the great difference in their WAI, WSI and SP values. It also explains the different effects that protein addition and microwave treatment had on them. The WAI and SP values of native rice were almost twice those of native potato starch. The low swelling power in potato starch can result from formation of stable amylose–phosphate group complexes (Kong et al., 2015) and from

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the weak internal organization caused by negatively-charged phosphate groups within the potato starch granules (Singh et al., 2003).

Adding proteins to native rice starch did not greatly influence WAI and SP values. However, MWT significantly decreased swelling power and ability to maintain gel structure after centrifugation. WAI decreases from MWT were 45% in non-protein rice starch, and 30% and 45% in CA- and SPI-rice starch blends, with respect to untreated counterparts. This WAI decrease could be attributed to increased crystallinity and interactions between amylose and amylopectin molecules strengthening intramolecular bonds, the formation of amylose-lipid complexes and crystalline region rearrangement resulting from HMT treatment (Zavareze and Dias, 2011).

Table 1. Effect of microwave treatment and protein presence on the hydration properties of starch samples. All values refer to sample dry matter.

Starch	**Protein**	**MW treatment**	**WAC (g/g)**		**WAI(g/g)**		**WSI(g/100g)**		**SP(g/g)**	
Rice	0	0	1.15	ab	13.42	cd	2.77	a	13.76	cd
		1	1.57	c	7.29	a	2.97	a	7.50	a
	CA	0	1.04	a	12.98	c	4.55	b	13.52	c
		1	0.91	a	9.00	b	6.92	c	9.62	b
	SPI	0	1.41	bc	13.77	d	2.94	a	14.14	d
		1	1.49	c	7.57	a	3.08	a	7.80	a
Analysis of variance and significance (p-values)										
Factor 1 (protein type)			***		**		***		***	
Factor 2 (MW treatment)			ns		***		***		***	
Factor 1x2			*		***		***		***	
Potato	0	0	0.91	a	6.75	a	3.57	b	6.96	a
		1	1.98	c	9.50	d	0.99	a	9.59	d
	CA	0	0.91	a	7.44	b	6.52	c	7.88	b
		1	1.27	b	8.72	c	3.12	b	8.97	c
	SPI	0	1.29	b	8.39	c	6.40	c	8.88	c
		1	2.77	d	9.37	d	1.15	a	9.47	d
Analysis of variance and significance (p-values)										

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Factor 1 (protein type)	***	***	***	***
Factor 2 (MW treatment)	***	***	***	***
Factor 1x2	***	***	***	***

Protein: 0: without protein; CA: 5% calcium caseinate; SPI: 5% soy protein isolate. MW treatment: 0: without treatment; 1: with treatment. WAC: Water absorption capacity; WAI: Water absorption index; WSI: Water solubility index; SP: Swelling power. The different letters in the corresponding column within each starch type indicate statistically significant differences between means at $p<0.05$. Analysis of variance and significance: *** $p<0.001$. ** $p<0.01$. * $p<0.05$. ns: not significant.

In contrast to what occurred in rice starch, WAI and SP values increased (10% and 24% for CA and SPI, respectively) when protein was added to potato starch. Similar results were observed by Chinma et al. (2003) when soy protein concentrate was added to another tuber starch such as tapioca. They attributed this increase to the reduced amylose content of the mixture from the decrease in the starch content, as amylose acts as a diluent and inhibits swelling. MWT also increased the WAI and SP in our potato samples. The largest increase was seen in protein-free potato starch, although WAI and SP also rose in protein-fortified samples as a result of MWT (41% [protein-free] versus 17% [CA] and 12% [SPI]). These increases could be related to the decrease already described in the potato starch amylose content from MWT and the dissociation of phosphate groups and amylose complexes through the high temperatures reached during MWT (Thomas and Atwell, 1999).

Water solubility index (WSI) represents the amount of solubilized starch molecules and is often used as an indicator of degradation and dextrinization of starch molecules (Jogihalli et al., 2017). Native potato starch WSI was significantly (data not shown) higher than that of rice. MWT did not affect solubility in protein-free rice starch, while it markedly decreased it in potato starch (Table 1). Similar results were reported by Nadiah et al. (2015) from microwaved potato and tapioca starches. The interaction between amylose and amylopectin branches detected in potato starch and the consequent denser granule structure formed would explain the drop in potato starch solubility. How adding protein affected WSI depended on the nature of the protein and

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also on the type of starch. Using a soluble form of casein, such as calcium caseinate, increased the solubility of the mixtures with both starches, (with increases of 64% and 82% for rice and potato starches, respectively). The solubility increased even more after MW treatment of the rice starch-CA blend (52%). However, adding SPI protein only increased native potato starch WSI (85%), while had no effect on rice starch. MWT decreased the solubility of protein-enriched potato starch blends, leading to WSI reductions of 52% (CA) and 82% (SPI) (Table 1). These decreases indicate that MWT alters the interactions between potato starch and proteins, greatly reducing the water solubility of the blends.

3.3. Viscometer profile

Pasting viscosity profiles of rice and potato starches, both native and microwave-treated, with and without proteins are shown in Fig. 1 and the results are summarized in Table 2. Microwave treatment greatly changed the pasting profile of the samples, which are likewise affected by starch granule size and amylose, lipid and phosphorous content (Jane et al., 1999). Pasting curves reflect the molecular phenomena that happen in starch granules during the heating cycle and provide a means of comparing the behaviour of potato and rice starches during cooking. The differences in pasting temperature between starches (Table 2) was due to the characteristic absence of lipids and phospholipids and the lower degree of crystallinity of tuber starches compared to cereals (Jane et al., 1999). In particular, MWT increased the potato starch PT by 27% in our study. Nadiah et al. (2015) also reported increased PT values for MW-heated potato and tapioca starches. The rise of PT (also found in HMT processes) has been associated to the fact that stronger bonds and cross-links between chains appear within the starch granule during MWT, requiring a higher temperature for structural disintegration and paste formation (Zavareze and Dias, 2011). No statistical differences in PT were observed

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between the native and the MW-treated rice starch samples, in contrast to what was previously observed with rice flour (Villanueva et al., 2018a), where PT increased ~10ºC. No PT changes were obtained either when glutinous rice starch was processed by dry heating at 130ºC (Qin et al., 2016). Puncha-arnon and Uttapap (2013) also found that treating rice starch with heat and moisture (at 100ºC and 20% moisture content for 16 h) increased PT only slightly, in contrast to the effect observed in treated-rice flour. Native potato starch had a very high PV because of its high phosphate monoester content and long branch chains (Jane et al., 1999). The lower PV observed in rice starch is related to its high concentration of lipids and phospholipids linked to amylose and long chains of amylopectin that restrict swelling of granules (McPherson, 1999). We found a significant decrease in PV in both native starches when proteins were added (Table 2). Ronda et al. (2014) observed that the reduction of starch due to the replacement of proteins caused lower pasting viscosity values. These proteins can retain water from the starch granules and consequently reduce initial starch granule swelling. Applying MWT also reduced potato starch PV, from 6.88 to 1.85 Pa·s. This result has previously been reported for both potato flour and potato starch; it was attributed to a possible reduction of amylose leaching as result of MWT (Varatharajan et al., 2010). The opposite effect was observed in rice starch, with a slight although significant increase in PV (7%) after treatment. Other studies reported significant PV decreases when rice flour was treated with HMT (Puncha-arnon and Uttapap, 2013) or MW radiation (Villanueva et al., 2018a). Proteins must play an important role in the effect of thermal treatments on rice flour. They can be denatured by heat treatment and some changes or interactions might occur between them during heat treatment (Puncha-arnon and Uttapap, 2013). Qiu et al. (2016) observed an increase in PV when a dry heating treatment was applied to rice flour or starch. The fact that MW treatment combines a

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high humidity heating period with a dry heating period could explain the intermediate behaviour that we observed in rice starch with respect to that obtained by Puncha-arnon and Uttapap (2013) and Qiu et al. (2016).

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Table 2. Effect of microwave treatment and protein presence on the pasting properties of starch samples.

Starch	Protein	MW treatment	PT(°C)		PV (Pa·s)		TV (Pa·s)		BD (Pa·s)		FV (Pa·s)		ST (Pa·s)		Peak time (s)	
Rice	0	0	74.6	ab	2.55	c	1.25	c	1.30	b	2.24	c	0.99	d	665	d
		1	73.9	a	2.73	d	1.23	c	1.49	c	2.29	c	1.06	e	627	ab
	CA	0	75.8	b	2.09	a	1.02	a	1.08	a	1.82	a	0.80	b	650	c
		1	74.6	ab	2.27	b	1.11	b	1.16	a	2.00	b	0.88	c	635	b
	SPI	0	73.9	a	2.32	b	1.03	a	1.30	b	1.72	a	0.69	a	618	a
		1	74.3	a	2.68	cd	1.35	d	1.33	b	2.31	c	0.96	d	620	a
Analysis of variance and significance (p-values)																
Factor 1 (protein type)			*		***		***		***		***		***		***	
Factor 2 (MW treatment)			ns		***		***		**		***		***		***	
Factor 1x2			ns		ns		***		ns		***		***		**	
Potato	0	0	70.0	a	6.88	e	2.06	b	4.82	d	2.84	ab	0.78	a	358	a
		1	76.2	b	1.85	b	2.11	b	0.00	a	3.70	b	1.59	b	687	c
	CA	0	71.4	a	3.15	d	1.66	a	1.49	c	2.34	a	0.67	a	518	b
		1	79.0	c	1.60	a	1.59	a	0.00	a	2.43	a	0.84	a	860	d
	SPI	0	70.5	a	2.72	c	1.50	a	1.22	b	2.39	a	0.89	ab	538	b
		1	78.1	c	1.92	b	2.01	b	0.00	a	3.29	ab	1.28	ab	762	cd
Analysis of variance and significance (p-values)																
Factor 1 (protein type)			**		***		**		***		*		ns		**	
Factor 2 (MW treatment)			***		***		*		***		*		*		***	
Factor 1x2			ns		***		**		***		ns		ns		ns	

Protein: 0: without protein; CA: 5% calcium caseinate; SPI: 5% soy protein isolate. MW treatment: 0: without treatment; 1: with treatment.
PT: pasting temperature, PV: peak viscosity, TV: trough viscosity, BD: breakdown, FV: final viscosity, ST: setback.
The different letters in the corresponding column within each starch type indicate statistically significant differences between means at $p<0.05$.
Analysis of variance and significance: *** $p<0.001$. ** $p<0.01$. * $p<0.05$. ns: not significant.

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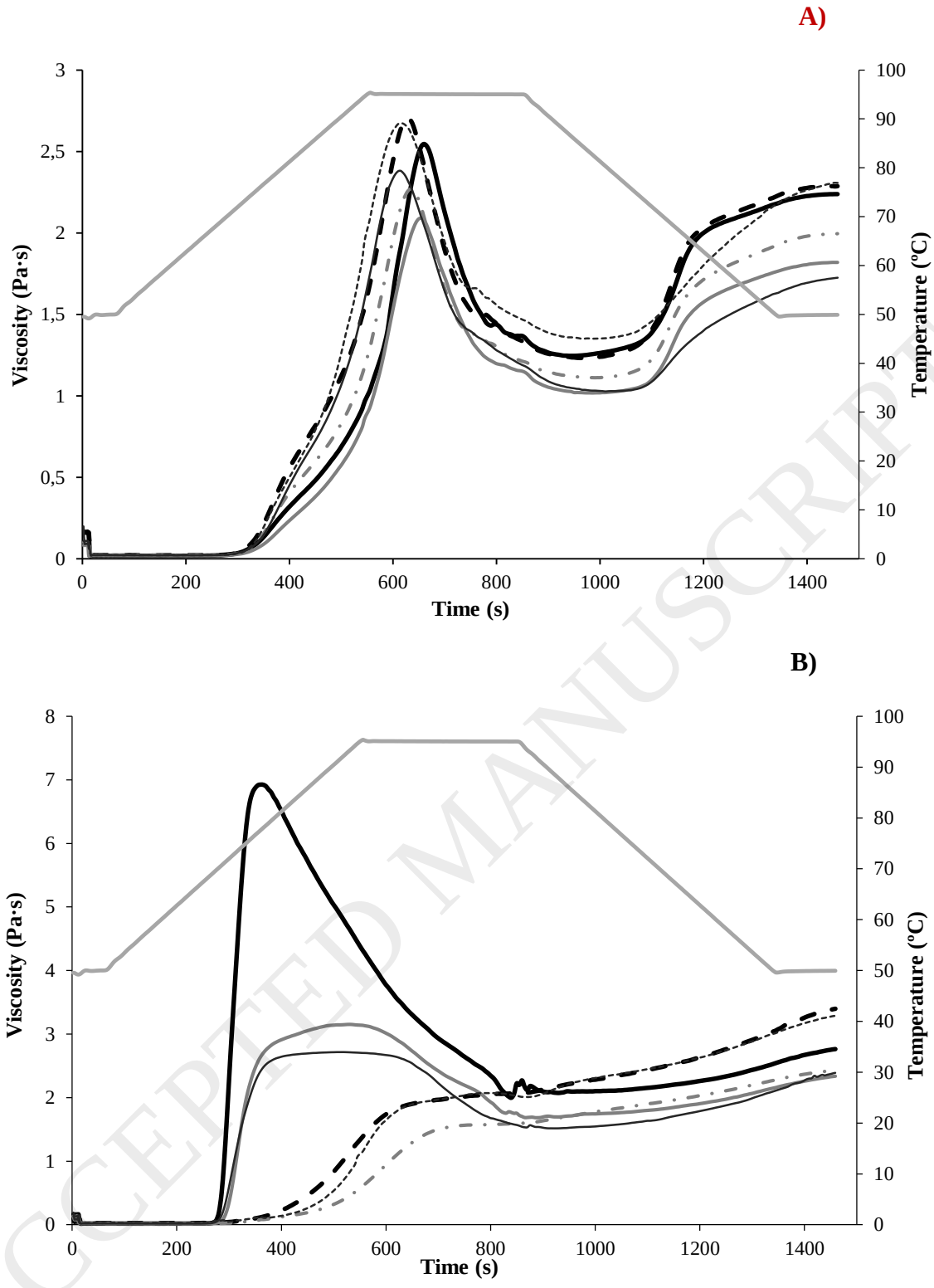

Figure 1. Pasting profiles of native and modified samples of rice starch (A) and potato starch (B). Starch without protein are represented by ——, with 5% calcium caseinate by ——, and with 5% soy protein isolate by ——. Microwave-treated protein-free samples are represented by – – –, microwave-treated samples with 5% calcium caseinate by - - - and with 5% soy protein isolate by -------. The temperature profile is represented by —— in the second axis.

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The low peak time of native potato starch increased significantly with MWT, reaching the peak values of rice starch. Native potato starch showed a much higher BD than rice starch, 4.82 versus 1.30 Pa·s (Table 2). A low BD value means high paste stability versus heat and shear, and is related to granule rigidity and high lipid content (Singh et al., 2003). Adding protein increased the paste stability of potato starch, decreasing the BD value by 69% and 75% for CA and SPI, respectively. However, in the case of rice starch, only CA had an effect and it was much slighter. MWT hardly affected the BD value of the rice starch samples. However, the treatment did reduce potato starch BD to zero, regardless of the presence of proteins. This reflects the ability of MWT to increase potato starch gel stability versus heating and shearing. This behaviour has already been observed in tuber starches (potato and tapioca) when they were moistened and thermally modified either by convection heat transfer (Klein et al., 2013) or MW radiation (Nadiah et al., 2015). ANOVA analysis (Table 2) revealed that protein type and MW treatment had a singularly strong effect on PT, PV and BD values of rice starch, without any mutual interaction of these factors. However, potato starch was highly susceptible, not only to protein type supplementation or MWT but also to the double interaction of the two factors on PV and BD.

The final viscosity (FV) of the gels formed after subsequent cooling decreased in presence of either animal or vegetal protein. The same had previously been reported by Ronda et al. (2014). The effect was more pronounced for rice starch gels than for potato gels. MWT increased the FV of rice+protein gel, while potato starch FV was unaffected. ANOVA analysis showed that each of the main factors influenced final viscosity, but the double effect was found only in rice starch.

Setback viscosity (ST), mainly related to the amylose leached from starch granules and their tendency to reorganise after gelatinisation (Miles et al., 1985), was equivalent for both native starches in our study. Adding proteins significantly ($p<0.001$) decreased this

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value in rice starch blends, while potato values remained unaffected. MWT increased ST in all rice starch-based formulation and protein-free potato starch. In the case of potato starch the increase was from 0.78 to 1.59 Pa·s. The ANOVA results confirmed that the simple and double-factor effects on rice starch ST were highly significant ($p < 0.001$). However, except for MWT, there were almost no effects on potato starch.

3.4. Gel rheological properties

The viscoelastic properties of gel samples were studied by dynamic oscillatory tests, applying a sinusoidal stress to the samples. The obtained viscoelastic moduli as a function of stress are shown in Figure 2. The parameters obtained from fitting the mechanical spectra to power law are summarized in Table 3. The high R^2 values obtained, always above 0.998 for all samples, demonstrate that the systems studied adjusted well to the power law model. Stress sweeps made it possible to establish the linear viscoelastic region (LVR) by identifying the maximum stress (τ_{max}) that samples could tolerate conserving their structure. These values are also included in Table 3. The elastic modulus, G', of gels made from untreated starches started to decrease at 108 and 267 Pa for rice and potato starch, respectively. This indicated that starch sample structures had different resistances to stress-induced rupture. According to Villanueva et al. (2018b), potato gels preserved their structure beyond rice starch gels, meaning that potato gels had good mechanical resistance and hardness. Adding proteins to rice starch gels reduced their resistance to breakage and led to values of τ_{max} 36% and 49% lower than those of protein-free gels for CA and SPI, respectively. The effect on potato starch gels differed depending on protein type: CA decreased τ_{max} by 19%, while SPI increased it by 14%, compared to protein-free potato gels. MWT increased the strength of rice gels, although this effect was only significant in SPI-enriched rice gels. The effect of MW radiation on potato gels was the opposite and stronger, being much more

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remarkable in the presence of proteins: τ_{max} decreased by 60% in gels without protein, 88% in CA protein-enriched gels and 72% in SPI-enriched gels.

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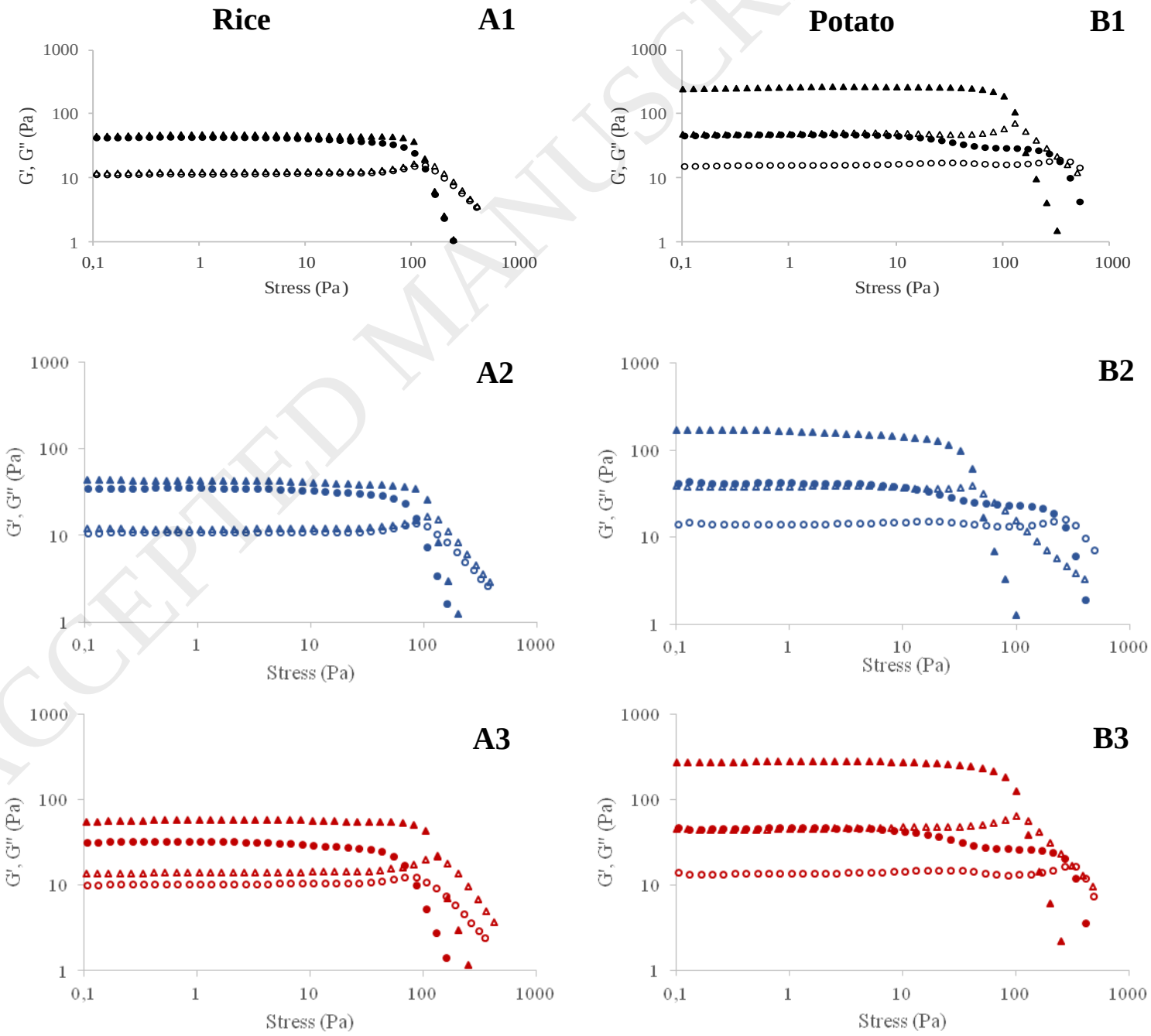

Figure 2. Strain sweeps of rice (A) and potato (B) starch gels without protein addition (A1 and B1), with 5% calcium caseinate (A2 and B2) or 5% soy protein isolate (A3 and B3). Non-treated samples are represented with circles and MW-treated samples with triangles. The elastic modulus, G', is represented using solid symbols and the viscous modulus, G'', with open symbols.

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Table 3. Effect of microwave treatment and protein presence on the dynamic rheological characteristics of starch gels.

Starch	Protein	MW treatment	G'_1 (Pa)		a		G''_1 (Pa)		b		$(\tan\delta)_1$		c		τ_{max} (Pa)	
Rice	0	0	52	ab	0.17	b	14	ab	0.36	a	0.27	bc	0.18	ab	108	c
		1	74	bc	0.15	a	17	ab	0.35	a	0.23	ab	0.20	b	119	c
	CA	0	41	a	0.18	b	12	a	0.36	a	0.29	c	0.18	a	69	ab
		1	47	a	0.17	b	13	ab	0.35	a	0.27	bc	0.18	ab	85	b
	SPI	0	39	a	0.19	b	12	a	0.36	a	0.30	c	0.17	a	55	a
		1	88	c	0.14	a	19	b	0.35	a	0.22	a	0.20	b	105	c
Analysis of variance and significance (p-values)																
Factor 1 (protein type)			ns		ns		ns		ns		ns		ns		**	
Factor 2 (MW treatment)			**		**		*		ns		**		*		**	
Factor 1x2			ns		ns		ns		ns		ns		ns		*	
Potato	0	0	53	a	0.181	e	16	a	0.29	b	0.31	e	0.11	ab	267	d
		1	421	d	0.077	a	51	b	0.22	a	0.12	a	0.15	c	101	b
	CA	0	46	a	0.184	e	14	a	0.28	b	0.31	e	0.10	a	216	c
		1	171	b	0.141	c	39	b	0.28	b	0.23	c	0.14	bc	26	a
	SPI	0	53	a	0.164	d	14	a	0.28	b	0.27	d	0.12	abc	304	d
		1	309	c	0.099	b	48	b	0.23	a	0.15	b	0.14	bc	84	b
Analysis of variance and significance (p-values)																
Factor 1 (protein type)			***		***		ns		*		***		ns		**	
Factor 2 (MW treatment)			***		***		***		**		***		**		***	
Factor 1x2			***		***		ns		*		***		ns		ns	

Protein: 0: without protein; CA: 5% calcium caseinate; SPI: 5% soy protein isolate. MW treatment: 0: without treatment; 1: with treatment.

G'_1, G''_1, and $(\tan\delta)_1$, represent the elastic and viscous moduli and the loss tangent at a frequency of 1 Hz. The a, b and c exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency, ω. τ_{max} represents the maximum stress that GF matrices can tolerate in the LVR.

The different letters in the corresponding column within each starch type indicate statistically significant differences between means at $p<0.05$.

Analysis of variance and significance: *** $p<0.001$. ** $p<0.01$. * $p<0.05$. ns: not significant.

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In all samples, G' and G" increased with frequency, G' being higher than G", which indicated that elastic character prevailed over viscous factor (Xie et al., 2013). The gel viscoelastic moduli G_1' and G_1'' and the loss tangent at 1 Hz were the same for the two native starches (potato and rice) regardless of protein presence or type. However, MWT always increased gel consistency, this impact was dependent on protein presence and type and starch type. The effect of MWT on gel rheological properties was also strongly dependent on the botanical origin of the starches. Both viscoelastic moduli increased in MW-treated potato starch samples, while only SPI addition affected the moduli in treated rice ones. The G_1' and G_1'' increases from MWT of potato gels were related to the gelatinization level and the network development of chains leached from the starch granules during the MW treatment (Xie et al., 2013). As a consequence of MWT, the increase in the elastic modulus was always more noticeable than in the viscous modulus. Consequently, MWT was always accompanied by a drop in the loss tangent, which meant a reinforcement of the elastic character of gels made from MW-treated starches or starch+protein blends compared to the untreated counterparts. Similar results have been reported for thermally-modified potato starch (Gryszkin et al., 2014). Tan δ value was very low for all samples (always < 0.4), which corresponded with a well cross-linked network. Adding SPI decreased the gel loss tangent of potato starch, but rice gels were unaffected by protein addition. Villanueva et al. (2018b) reported that the rheological properties of rice and potato starch gels fortified with egg albumen or SPI depended significantly on the double interaction (starch x protein), concluding that the same protein exerted a different effect depending on the starch source. The values of the "a" exponent were always below the exponent "b". This meant that G'' increased with frequency faster than G'. This yielded an increase in tan δ with frequency, which could also be confirmed from the positive value of exponent "c" (Table 3). Consequently, gels increased their viscous behaviour with frequency.

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4. Conclusions

MWT has been shown to be successful in modifying starches and their protein mixtures and has proven to be efficient in altering the physical properties of products made from them. It is particularly useful in the development of protein-enriched GF products. The results showed that the effect of MWT is dependent on starch source and protein type. MWT changed the hydration properties and enhanced water absorption index and swelling power in potato samples, while it decreased them in rice starch samples, regardless of type of protein added. MW radiation influenced mainly the pasting properties of potato starch, increasing pasting temperature and setback, and decreasing peak viscosity and breakdown. The treatment increased the viscoelastic moduli significantly and decreased the loss tangent of potato gels both with and without proteins. In general, the inclusion of proteins increased WAC, WAI, SP and WSI, decreased the viscosity of gels and increased their stability, being the effect more marked for SPI incorporation.

This research helps understand the changes that occur during MW treatments of model systems and can help design and improve the quality of new products. MW-assisted heating is an innovative flour treatment method and can be used as an alternative to chemical modification.

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